Crystal Structure of the Catalytic Portion of Human HMG-CoA Reductase

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Beamline(s): **X12B**

Introduction: 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) catalyzes the formation of mevalonate, the committed step in the biosynthesis of sterols and isoprenoids. This reductive cleavage of HMG-CoA to mevalonate utilizes two molecules of NADPH. The activity of HMGR is controlled through synthesis, degradation and phosphorylation to maintain the concentration of mevalonate-derived products. In addition to the physiological regulation of HMGR, the human enzyme has been targeted successfully by drugs in the clinical treatment of high serum cholesterol levels.

Methods and Materials: Three crystal structures were determined with different substrates bound to the protein: form A contains HMG and CoA (refined to 2.1 Å resolution), form B (refined to 2.8 Å resolution) contains HMG-CoA, and form C (refined to 2.0 Å resolution) contains HMG, CoA, and $NADP^+$. The structure of form A crystals was determined by multi-wavelength anomalous dispersion (MAD) from 58 selenomethionine-substituted positions. 45 of the selenium positions were determined using direct methods implemented in the program Shake-n-Bake. The remaining 13 selenium positions were located with difference Fourier methods. The structures of crystal forms B and C were determined by molecular replacement using the structure of the A form as the search model.

Results: Catalytic portions of human HMGR form tetramers with approximate D2-symmetry and overall dimensions of roughly 110 Å X 80 Å X 70 Å. The individual monomers wind around each other in an intricate fashion (Figure 1). In the tetramer the monomers are arranged in two dimers, each of which has two active sites. The active sites are formed by residues from both monomers. CoA makes numerous contacts with the large domain, while $NADP^+$ is predominately bound to the small domain of the monomer. The HMG binding pocket is located between the large and the small domain. The most important structural element in the binding of HMG is a loop that contains a *cis*-peptide bond between residues C688 and T689.

The formation of the HMGR tetramer buries a total solvent-accessible area of 24,260 Å2 or 46% of the tetramer surface. Ultracentrifugation experiments indicate that the catalytic portion of the protein is tetrameric in solution as well as in the crystal. The membrane domain of human HMGR is responsible for the enhanced degradation of HMGR in response to increased concentrations of oxysterols. The crystallographic data suggest that the soluble domains may initiate the tetramerization of the membrane domains, and that dissociation of the membrane domains increases the accessibility of HMGR for the protease cleavage, resulting in the inactivation of the enzyme.

Analysis of the three structures of human HMGR provides new insights into the mechanism by which the reductive cleavage is catalyzed. The reaction intermediate mevaldyl-CoA has a negatively charged oxygen and must be stabilized in the enzyme. This is accomplished by K691, whose side chain is ideally positioned in the middle of the active site. The ordering of C-terminal residues, including H866, upon $NADP^+$ binding results in the completion and closure of the active site and H866 moves within H-bonding distance from the thiol. The OE1 atom of E559 is 2.6-2.7 Å away from the HMG carbonyl oxygen and 3.5-3.7 Å from D767 (atom OD2). The proximity of E559 to D767 could potentially raise the pKa of the glutamic acid side chain such that it may be protonated. Consequently, we propose that E559 is the proton donor for mevaldehyde. D767 is critical in the catalysis, because its side chain is positioned near D559 and it also forms ionic interactions with K691, stabilizing the lysine side chain in the active site.

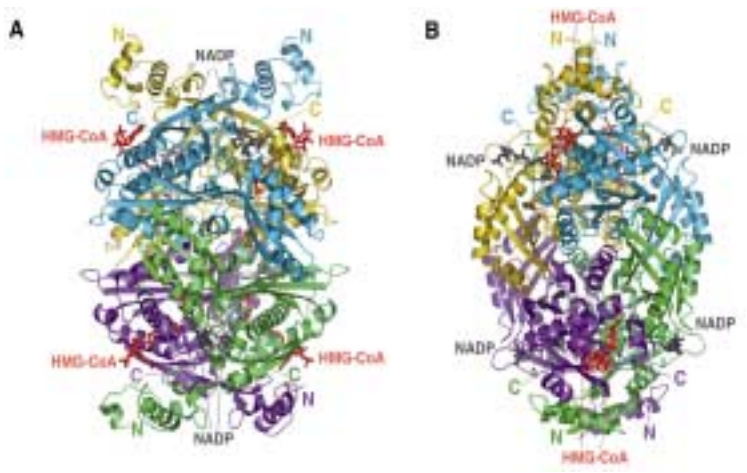

Figure 1. The HMGR tetramer seen from the front (A) or the side (B).

Acknowledgments: The MAD data used to solve the form A structure were collected at APS SBS ID-19. The high resolution form A native data were collected at NSLS Beamline X12B. The form C data were collected at SSRL Beamline 7-1.

References: E.S. Istvan, M. Palnitkar, S.K. Buchanan, J. Deisenhofer, "Crystal Structure of the Catalytic Portion of Human HMG-CoA Reductase: Insights into Regulation of Activity and Catalysis," EMBO J. **19**, *5*, p. 819-830, 2000.
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